

WHAT IS CLAIMED IS:

- 1                   1.    A constitutively active  
2   phosphatidylinositol 3-kinase polypeptide comprising p85  
3   subunit iSH2 domain sequences linked at the carboxy-terminus  
4   by a linker to the amino-terminus of a p110 subunit.
  
- 1                   2.    The polypeptide of claim 1, wherein the N-  
2   terminal 20 amino acids are eliminated from the p110 subunit.
  
- 1                   3.    The polypeptide of claim 1, wherein the iSH2  
2   domain sequences consist essentially of amino acids 466 to 567  
3   of the p85 subunit.
  
- 1                   4.    The polypeptide of claim 1 further comprising a  
2   tag at the amino or carboxy terminus.
  
- 1                   5.    The polypeptide of claim 4, wherein the tag is  
2   an epitope.
  
- 1                   6.    The polypeptide of claim 5, wherein the epitope  
2   tag is a *myc* epitope at the carboxy terminus of the p110  
3   subunit.
  
- 1                   7.    A constitutively active phosphatidylinositol 3-  
2   kinase polypeptide, comprising amino acids 466 to 567 of the  
3   p85 subunit iSH2 domain linked by a 10 amino acid glycine  
4   kinker to a p110 subunit at the amino-terminus of the p110  
5   subunit, and a *myc* epitope as defined by SEQ ID NO. \_\_\_\_  
6   (EQKLISEEDL) fused to the carboxy terminus of the p110  
7   subunit.
  
- 1                   8.    An expression vector comprising a DNA sequence  
2   encoding a polypeptide according to claim 1.
  
- 1                   9.    An expression vector comprising a DNA sequence  
2   encoding a polypeptide according to claim 4.

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1           10. The vector of claim 9, also comprising pGC  
2 plasmid sequences.

1           11. An expression vector comprising a DNA sequence  
2 encoding a polypeptide according to claim 7, said vector being  
3 the plasmid pCG-pl10·myc.

          12. A cell containing the expression vector of  
claim 8 or 11.

1           13. A method of producing a constitutively active  
2 phosphatidylinositol 3-kinase polypeptide, comprising  
3 introducing the expression vector of claim 8 or 11 into a host  
4 cell under conditions which favor expression of the  
5 polypeptide and isolating the resultant expressed polypeptide.

1           14. The method of claim 13, wherein the host cell  
2 is selected from the group consisting of a smooth muscle cell,  
3 a 293 cell, and a CHO cell.

1           15. The method of claim 13, wherein the host cell  
2 is a mammalian fibroblast cell.

1           16. The method of claim 15, wherein the fibroblast  
2 cell is selected from the group consisting of a COS 7 cell, a  
3 NIH 3T3 cell and a rat 3YI cell.

1           17. A method of producing an inositol phosphate  
2 product comprising reacting a phosphatidylinositol 3-kinase  
3 polypeptide of claim 1 or 7 with a phosphoinositide lipid  
4 substrate under appropriate kinase reaction conditions, and  
5 isolating the resultant product.

1           18. The method of claim 17, wherein the product is  
2 phosphatidylinositol 3'-phosphate (PI 3'-P) and the lipid  
3 substrate is phosphatidylinositol (PI).

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19. The method of claim 17, wherein the product is phosphatidylinositol 3',4'-bisphosphate (PI 3',4'-P<sub>2</sub>) and the lipid substrate is phosphatidylinositol 4'-phosphate (PI 4'-P).

20. The method of claim 17, wherein the product is phosphatidylinositol 3',4',5'-phosphate (PI 3',4',5'-P<sub>3</sub>) and the lipid substrate is phosphatidylinositol 4',5'-bisphosphate (PI 4',5'-P<sub>2</sub>).

21. A kit for preparing an inositol phosphate product, comprising:  
a constitutively active phosphatidylinositol 3-kinase polypeptide of claim 1 or 7; one or more phosphoinositide substrates; and instructions for preparing the inositol phosphate reagent.

22. The kit of claim 21, further comprising a buffer for reconstituting said phosphatidylinositol 3-kinase polypeptide and a reaction buffer.

23. The kit of claim 21, wherein the inositol phosphate product is selected from the group consisting of PI 3-P, PI 3',4'-P<sub>2</sub>, and PI 3',4',5'-P<sub>3</sub>.

24. The kit of claim 21, wherein the phosphoinositide substrate is selected from the group consisting of PI, PI 4'-P, and PI 4',5'-P<sub>2</sub>.

25. A method of identifying a cellular target protein substrate of a phosphatidylinositol 3-kinase, said method comprising the steps of:

- (a) providing a phosphatidylinositol 3-kinase polypeptide of claim 1;
- (b) providing a test cell lysate thereof;
- (c) providing a negative control cell lysate not contacted with said phosphatidylinositol 3-kinase polypeptide;

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9 (d) contacting said phosphatidylinositol 3-kinase  
10 polypeptide with said test cell lysate in the presence of  
11 labeled ATP under conditions which allow said  
12 phosphatidylinositol 3-kinase to phosphorylate any cellular  
13 target protein present in said cell lysate to form a  
14 phosphorylated target protein; and

15 (e) comparing said test cell lysate with said  
16 negative control lysate to detect said phosphorylated target  
17 protein in said test cell lysate and thereby identifying the  
18 cellular target protein substrate.

1 26. The method of claim 25, wherein the ATP is  
2 [ $\gamma^{32}\text{P}$ ]ATP.

1 27. The method of claim 25, wherein both cell  
2 lysates are from stimulated cells.

1 28. The method of claim 26, wherein said  
2 phosphorylated target protein is detected by:

3 performing SDS-PAGE on said cell lysates after step  
4 (d) to separate proteins contained therein, on a gel matrix;  
5 and

6 preparing an autoradiograph of said gel to detect  
7 radiolabeled bands present in the test cell lysates, but  
8 absent from the negative control lysate, as indicative of the  
9 presence of said phosphorylated target protein in the test  
10 cell lysate.

1 29. A method for identifying an associating protein  
2 of an active phosphatidylinositol 3-kinase, comprising:

3 expressing a constitutively active  
4 phosphatidylinositol 3-kinase polypeptide of claim 1 or 7 in a  
5 mammalian cell under conditions which favor expression of the  
6 polypeptide;

7 biosynthetically labeling proteins of said active  
8 kinase expressing cell to produce labeled proteins;

9 obtaining a lysate from said cell;

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10 immunoprecipitating said expressed  
11 phosphatidylinositol 3-kinase polypeptide from said cell  
12 lysate to produce an immunocomplex;  
13 detecting a labeled protein that co-  
14 immunoprecipitates with said phosphatidylinositol 3-kinase  
15 polypeptide, within said immunocomplex, wherein said co-  
16 immunoprecipitating protein is considered an associating  
17 protein.

1 30. The method of claim 29, wherein said co-  
2 immunoprecipitating protein is detected by solubilizing said  
3 immunocomplex to release labeled proteins contained therein;  
4 separating said released proteins by SDS-PAGE; and performing  
5 autoradiography to detect a labeled protein other than said  
6 phosphatidylinositol 3-kinase polypeptide, said labeled  
protein being an associating protein.

1 31. A method for identifying an associating protein  
2 of an active phosphatidylinositol 3-kinase, comprising:  
3 exposing a phage or bacterial peptide library to a  
4 constitutively active phosphatidylinositol 3-kinase  
5 polypeptide of claim 1 or 7 to allow binding of a peptide from  
6 said library to said constitutively active  
7 phosphatidylinositol 3-kinase polypeptide to form a bound  
8 peptide; and  
9 isolating said bound peptide, wherein said bound  
10 peptide is considered an associating protein.

1 32. The method of claim 31, wherein said bound  
2 peptide is isolated by affinity purification.

1 33. A method of screening for an inhibitor of  
2 phosphatidylinositol 3-kinase activity, said method  
3 comprising:  
4 providing a constitutively active  
5 phosphatidylinositol 3-kinase polypeptide of claim 1 or 7;  
6 exposing one or more test compounds to said  
7 phosphatidylinositol 3-kinase polypeptide in the presence of a

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8 phosphatidylinositol 3-kinase substrate and [<sup>32</sup>P]ATP to allow  
9 phosphorylation of said substrate; and

10 assaying for the presence of phosphorylated  
11 substrate wherein the absence of phosphorylated substrate is  
12 indicative that the test compound is an inhibitor of  
13 phosphatidylinositol 3-kinase activity.

1 34. The method of claim 33, wherein said substrate  
2 is phosphatidylinositol and said phosphorylated substrate is  
3 phosphatidylinositol 3'-P.

1 35. A method of treating a disease selected from  
2 the group consisting of proliferative, inflammatory, allergic  
3 and cardiovascular diseases, comprising administering to a  
4 patient, a therapeutic formulation comprising an inhibitor of  
5 phosphatidylinositol 3-kinase activity in an amount effective  
6 to block phosphatidylinositol 3-kinase activity in affected  
7 cells of said patient.

1 36. The method of claim 35, wherein the  
2 proliferative disease is cancer or psoriasis.

1 37. A method of promoting wound healing, comprising  
2 administering to a patient, a therapeutic formulation  
3 comprising a phosphatidylinositol 3-kinase polypeptide of  
4 claim 1.

1 38. A formulation for treating a proliferative  
2 disease, comprising a therapeutically effective amount of an  
3 inhibitor of phosphatidylinositol 3-kinase.

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